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CRISPR-Cas9 Mediated Genome Editing a Promise to Cure HIV: A Systematic Review

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Abstract: Human Immunodeficiency Virus (HIV-1) infection might be controlled using long-term antiretroviral therapy. However, it causes various side effects rather than its cost-effectiveness. Thus, the development of a permanent cure for HIV-1 that most probably relies on gene therapy is highly required. Relying on that, genome editing approaches like Clustered Regularly Interspaced Short Palindromic Repeats associated with Cas9 (CRISPR-Cas9) have been utilized to target coreceptors, including CCR5 and CXCR4, hoping to find a curative method against HIV infection. Accordingly, the current study aims to systematically review CRISPR-Cas9 mediating genome editing studies, mainly CCR5 or CXCR4 or simultaneous genome editing to make HIV resistant primary human CD4+ T cells. A systematic review was conducted on original articles focusing on CRISPR-Cas9 mediated genome editing in HIV, published between 2015 and 2023. As a result, the CRISPR-Cas9 technology has demonstrated effective gene editing to confer HIV-1 resistance by targeting CCR5 and CXCR4 receptors in CD4+ T cells. Studies show that knockout of CCR5 or CXCR4, through CRISPR-Cas9 mediated frameshift insertions, base editing, and other genetic interventions, provides substantial resistance to HIV-1, with no major cytotoxic effects observed in primary T cells. Dual knockout of CXCR4 and CCR5 offers resistance to both X4- and R5-tropic HIV strains, suggesting enhanced protective potential. However, challenges remain, particularly due to CXCR4's crucial cellular roles, necessitating careful assessment of functional impacts and off-target effects. Additionally, leveraging natural mutations like CCR5Δ32 has inspired promising avenues for durable HIV-1 resistance. While targeting viral genomes, especially latent reservoirs, appears safer by avoiding host genome alterations, reinfection risks persist. These findings highlight the promise of CRISPR-based HIV therapies, though clinical translation will require rigorous optimization and evaluation.

Keywords: *CRISPR-Cas9; HIV; CCR5; CXCR4; Genome Editing.*

1. Introduction

The human immunodeficiency virus (HIV), after its confirmation to be a pathogenic cause of acquired immunodeficiency syndrome (AIDS), has become a global epidemic. The HIV infection can progressively lead to immunodeficiency and severe neurocognitive disorders, then results in fatal AIDS [1]. Despite decreasing viremia and extending the HIV infected individual's life span via highly active antiretroviral therapy (HAART), it has many limitations and side effects, including drug resistance and toxicity, besides high cost-effectiveness [2, 3]. Moreover, latent HIV infection via its reservoir would result in the virus rebound formerly the antiretroviral therapy is stopped [4]. Consequently, the development of alternative viral therapeutic approaches is an urgent need to overcome this challenge.

HIV-1 targets human $CD4^+$ T cells and depends on host factors for the infection and propagation, as it uses glycoproteins on its envelope to specifically bind chemokine receptors, including C-C Motif Chemokine Receptor 5 (CCR5) and C-X-C Motif Chemokine Receptor 4 (CXCR4) [5, 6]. Commonly, when HIV-1 infects human, the CXCR4 along with or as an alternative coreceptor to CCR5, is used for entry to the targeted cells. Moreover, in half of late-stage infections, the X4-tropic HIV-1 strains causes faster progression of the disease [7]. The individuals who rarely have a natural homozygous *CCR5-Δ32* mutation showed highly resistant to HIV-1 infection without having a noticeable abnormal phenotype, except for increasing susceptibility to some pathogens [8]. Accordingly, the ablation of CCR5 using zinc finger nucleases (ZFNs) in $CD4^+$ T cells resulted in

inhibition of HIV-1 infection [9]. Moreover, dual modification of *CCR5* and *CXCR4* in primary human $CD4^+$ T cells protected cells from R5 and X4 HIV-1 strains [9]. Recently, the CRISPR-Cas9 system has provided a new and alternative avenue with minimum off targeting for the purpose of gene editing in order to correct the errors, gene knockout or disruption [10].

CRISPR-Cas system is originally considered an adaptive immune system of bacteria and archaea. This system is mainly made of CRISPR RNAs (crRNAs) and CRISPR-associated proteins, which function to identify and degrade the invading viral genome [10]. The CRISPR system has shown to have high ability in gene editing of different host cells, including zebrafish, drosophila, mice, plant, monkeys, and even in human cells [11, 12]. In human CRIPER-Cas 9 has been utilized, hoping to treat various non-curative diseases, including neurodegenerative diseases, autoimmune diseases, diabetes, HPV, Hepatitis B and C virus, HIV, and even cancer [13-15]. Regarding HIV recently, CRISPR-Cas9 has been utilized to target CCR5 and CXCR4 in both single-targeting and simultaneous targeting [16-18]. Accordingly, the current study aims to systematically review the current state of CRISPR-Cas9-mediated genome editing and its potential roles in curing HIV. Moreover, we wanted to evaluate the safety, effectiveness, and challenges related to this therapeutic approach for HIV.

2. Methodology

2.1 Data Sources and Search Strategy

A systematic search was conducted on electronic databases including Web of Science, Scopus, PubMed, and Science Direct. The search was confined to studies published after 2015, and only titles and abstracts were considered. The search was restricted to articles written in English. The literature retrieval utilized the following keywords: CRISPR-Cas9, HIV, HIV-1, Gene therapy, Gene Editing, CCR5, CXCR4, Elimination of HIV genome, and latent HIV. Furthermore, we conducted a thorough manual search of the references cited in the included publications as well as pertinent published reports.

2.1.1 Inclusion and Exclusion Criteria

Only studies conducted between 2015 and 2023 and published in English, which focused on CRISPR-Cas9 mediated genome editing in HIV, were selected based on their titles and abstracts. Full texts were obtained for any studies that met the eligibility criteria. Any issue was handled by comprehensive deliberation, leading to a consensus. The eligibility of included articles were relied on PRISMA guidelines as shown in Figure 1. Every included article must adhere to the following criteria: (1) The genome editing must be done using CRISPR-Cas9. (2) The article must focus only on HIV. (3) Studies must be published in reliable journals. (4) Studies that used cell lines, model organism and human primary $CD4^+$ T cells are included. All studies that were not included have the following items: (1) Replicated articles; (2) papers published prior to 2015, correspondences, dissertations, conference summaries, case studies, meta-analyses, and review articles. (3) Studies published in languages other than English (4) Studies used editing tools rather than CRISPR-Cas9.

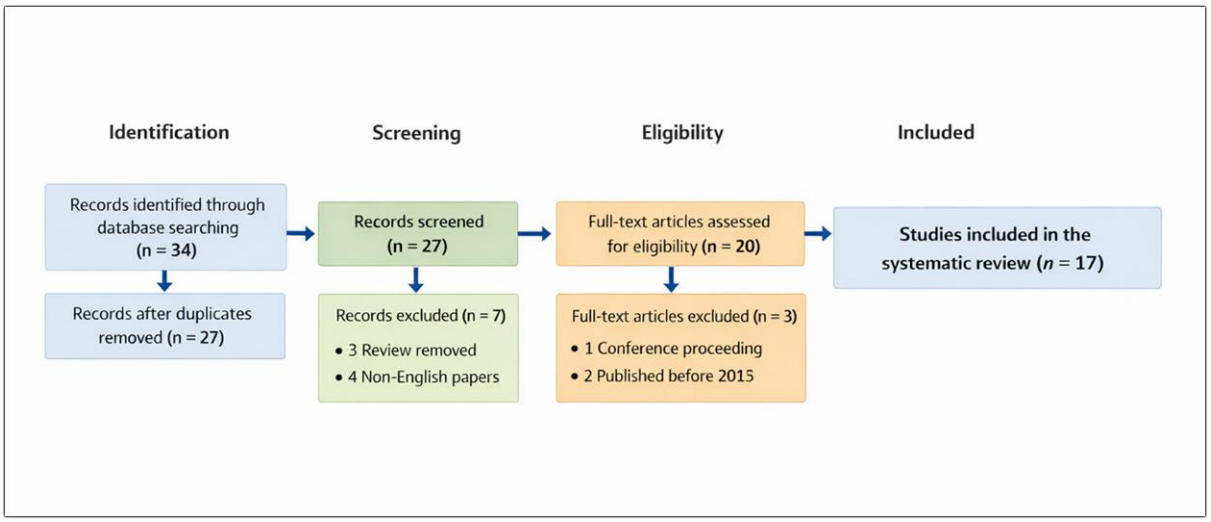

Figure 1: Summarizing the searching and excluding strategy

3. Targeted Possibility for the Therapy in the HIV Infection Cycle

The human immunodeficiency virus is a pathogenic cause of acquired immunodeficiency syndrome (AIDS). The cDNA of HIV consists of two long terminal repeat (LTR) structures adjoining the entire viral genome, which mainly consists of nine genes that are overlapped: gag, pol, env, tat, rev, nef, vif, vpr, vpu [19]. HIV infection can progressively lead to immunodeficiency and severe neurocognitive disorders, ultimately resulting in AIDS [1]. The HIV infection cycle is a complicated and organized series of events that facilitates the entry of the virus into the targeted cells, which are $CD4^+$ T cells and monocytes [20]. The HIV-1 uses glycoproteins on its envelope to specifically bind Cluster of Differentiation (CD)4 on the target cell [6], as indicated in Figure 2. Furthermore, for the process of viral binding, co-receptors CCR5 and CXCR4 also play a major role [5]. The viral binding would be followed by a fusion step, which is mainly facilitated by inserting glycoprotein gp41of the HIV envelope into the host cell membrane, that results delivery of the viral core, which contain genetic code, into the cell [21]. Consequently, Inhibitors targeting interactions of viral glycoproteins with cell receptor (CD4) and coreceptors (CCR5 and CXCR4) have emerged as promising therapeutic strategies to prevent viral entry and initial infection. However, due to its crucial cellular function that functions as an anchor for important kinases, CD4 is not a preferable and druggable target [22].

Once inside the host cell, the HIV genome, which consists of RNA, is reverse transcribed into cDNA using the viral reverse transcriptase enzyme. to allow insertion into the host genome controlled by integrase, another viral enzyme [21]. This inserted DNA will then be transcribed and translated into HIV proteins, in order to assemble new viral particles (Figure 2). These critical steps also provide other paths for therapeutic intervention. Reverse transcriptase inhibitors such as nucleoside and non-nucleoside analogs lead to disrupting this process by inhibiting the conversion of viral RNA into DNA, thereby impeding viral replication. Moreover, Inhibitors targeting integrase, known as integrase strand transfer inhibitors (INSTIs), have proven to be effective in preventing this integration, offering a targeted approach to stopping viral replication [23]. Following integration, several host proteins got hijacked for viral replication, protein synthesis, and assembly. Thus, protease inhibitors that act as another class of antiretroviral drugs result in disrupting the final stages of the viral life cycle by inhibiting the activity of the viral protease enzyme; in this way, the formation of matured and infectious viral particles is prohibited [24].

Although the targeted therapies have dramatically improved HIV management, challenges including drug resistance, potential side effects, and the persistence of viral reservoirs still persist [22]. Therefore, novel therapeutic strategies such as immune-based therapies and gene editing technologies might address these challenges and develop more potent and sustainable treatments. Especially, targeting

CCR5 and CXCR4 with the most prominent gene editing tools (ZFNs, TALENs, and CRISPR) might be the most curative target, mainly when combined with the fact that the CCR5Δ32/Δ32 mutation confers resistance to HIV infection [25]. Figure 2 summarizes the main steps of the HIV infection cycle that can be targeted for therapy.

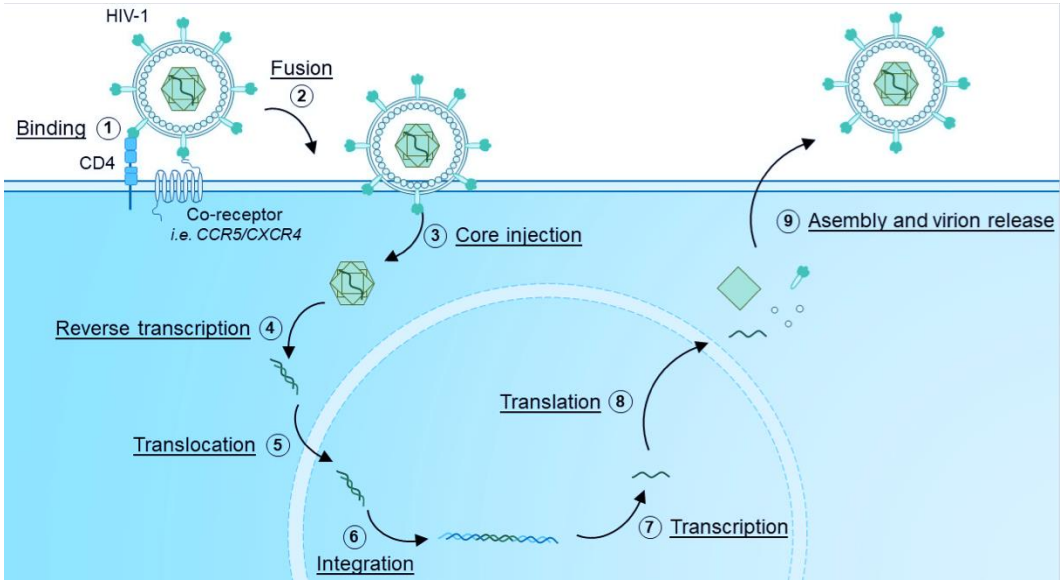

Figure 2: Summary of HIV infection and replication cycle [22].

4. CRISPR-Cas9:

The CRISPR-Cas9 system is originally considered as the bacterial adaptive immune system known as clustered regularly interspaced short palindromic repeats (CRISPR)-associated endonuclease protein 9 (Cas9), as indicated in Figure 3 [10]. On the other hand, it is considered a gene editing tool that works Watson-Crick base pairing principle. Besides, its easiness of use and precise genome targeting and edition and less off targeting results made CRISPR-Cas9 to be used more compared to previous gene editing tools [26].

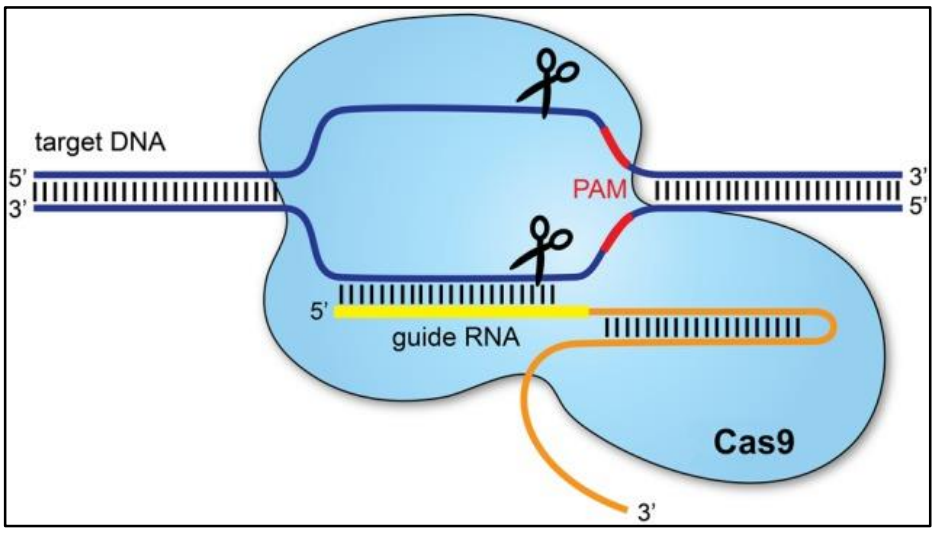

Figure 3: Structure of the CRISPR-Cas9 system, consisting of the Cas9 endonuclease and guide RNA.

To catalyze the DNA strand, CRISPR relies on an endonuclease protein (Cas9), which makes a double-strand break (DSB) at the targeted DNA sequence, which is guided by a 20-nucleotide sequence within

an associated CRISPR RNA (crRNA) transcript [27, 28]. To perform its activity, which is site-specific DNA recognition and cleavage, Cas9 needs crRNA and a trans-activating crRNA (tracrRNA) [28]. Various studies have demonstrated that it's possible to redesign the crRNA:tracrRNA complex as a single transcript (single-guide RNA or sgRNA), which has the ability to bind with Cas9 and target a recognition site of DNA [10, 28, 29]. Consequently, relying on the designed sgRNA, Cas9 can cleave dsDNA at any site that is defined by the guide RNA sequence and includes a GG protospacer-adjacent motif (PAM) [28, 30]. These findings have declared that Cas9: sgRNA complexes might serve as a simple and versatile RNA-directed system that allows generating DSBs, which can be used as an effective genome editing tool. Principally. the double strand breaks inside the cells, which are induced by Cas9: sgRNA complexes, enable either alteration (correction) of the targeted locus on DNA sequence via homology-directed repair (HDR) or gene knockout (disruption) by nonhomologous end joining (NHEJ) [30, 31].

In contrast to ZFNs and TALENs, which depend on proteins recoding using large segments of DNA (500–1500 bp) in order to target the new site. While CRISPR-Cas9, by changing only the 20-bp protospacer of the guide RNA and the unchanged Cas9 protein, can be easily adapted to target any genomic sequence. This way of easy manipulation and the property of RNA-dependent make CRISPR-Cas9 a better option than ZFNs and TALENs, especially in the case of multiplex gene editing, hence multiple gRNA can be designed to target more than one site or even more than one gene, resulting in simultaneous gene editing that is very difficult in the case of ZFNs and TALENs [31]. Therefore, CRISPR-Cas9 can be used to engineer precise deletions (mutate) multiple genes at once, although it should be noted that when simultaneously multiple copies of both ZFN and TALEN are used, the same outcomes might be achieved, but with many more challenges [32].

On the other hand, the big size of Cas 9 is considered a disadvantage of CRISPR-Cas9. For instance, the cDNA of *S. pyogenes* that encodes Cas9 is about 4.2 kb in size, making it larger than a TALEN monomer and much larger than a ZFN monomer. This size presents challenges in CRISPR-Cas9 system delivery using viral vectors, mainly due to the additional promoter and a polyadenylation sequence beside the gRNA molecule [33]. A lentivirus, which has a larger capacity for delivering compared to other viral vectors, can be used to deliver the S. pyogenes CRISPR-Cas9 system, while adeno-associated virus (AAV), with a limited cargo size of less than 5 kb, cannot accommodate the CRISPR-Cas9 in general. Although this challenge might be overcome as various CRISPR-Cas systems are available in other species, for example, the cDNA of *N. meningitidis* encoding Cas9 is approximately 3.2 kb in size, thus it might be delivered via AAV for different therapeutic applications [33].

Overall, considering the originality of the CRISPR-Cas9 system, variety in gRNA designing, and precise targeting, it can be considered a vital genome editing tool that might be used in curing various gene-based diseases.

5. CRISPR-Cas9 Mediating CCR5 Gene Editing

The HIV infection journey starts with its entry into the host cells. For this to happen, the viral glycoprotein (gp120) needs to interact with the CD4 receptor and a co-receptor CCR5 [6]. Thus, the CCR5 acts as the main coreceptor that facilitates CCR5-tropic HIV-1 (which is also considered as R5 variant) entry, has been targeted by various therapeutic approaches in HIV treatment in both human cell lines and animal models using different gene editing tools, including CRISPR-Cas9 [17, 34, 35]. Despite challenges including off-targeting, low frequency of gene editing, delivery method obstacles, and limited clinical application, CRISPR-Cas9 mediating genome editing of the CCR5 gene might be a curative key for HIV infection. Table 1 gives an inclusive summary of CCR5 gene editing in cell lines and human primary $CD4^+$ T cells.

In 2009, the case of Mr. Timothy Brown (Berlin patient) was reported by Dr. Hutter. The Berlin patient, who received allogeneic CCR5Δ32/Δ32 bone marrow transplantation, as he was suffering from HIV

infection as well as acute myeloid leukemia, received allogeneic CCR5-tropic HIV resistant [36]. Relying on that, various studies based on targeting the CCR5 gene were performed. For example, Guan [37] has demonstrated that CRISPR-Cas9 has efficiently edited and introduced various mutations that resulted in the knockout of the *CCR5* expression locus in cell lines. Furthermore, they transduced primary $CD4^+$ T-lymphocytes and disrupted expression of CCR5 by constructing chimeric Ad5F35 adenoviruses carrying CRISPR-Cas9 components, which resulted in HIV-1 resistance [37]. Moreover, another study has shown that the hematopoietic stem cells transplantation, which was genetically disrupted with CRISPR-Cas9 to be homozygous for a 32 bp deletion in CCR5 lead to loss of detectable HIV-1 [17]. Additionally, Scheller, Rashad [17] utilized biallelic frameshift insertion and determined that only individuals with a homozygous frameshifted mutation (CCR5-Δ32) confer complete resistance to HIV-1 infection. Consequently, as a curative solution for HIV infection, CCR5 gene editing has been targeted using different gene editing tools and, more recently, using CRISPR-Cas9 (summarized in Table 1).

Although the genetic knockout impacts as well as CRISPR-Cas9 accuracy should be carefully considered to ensure patient safety [25], hence, CCR5 has a critical role defensing other viruses [38]. For instance, it has been reported that the mutated CCR5 (CCR5Δ32/Δ32) leads to increasing the risk for symptomatic infection or fatal outcome in various viruses, including West Nile Virus and Influenza [38]. However, contradicting reports have been published in that regard, for example, the CCR5Δ32/Δ32 found to be associated with protection against hepatitis B infection [38]. In conclusion, based on the low natural occurrence of the CCR5 Δ32/Δ32 mutation and relying on the recent published works on CCR5 disruption and its impact on HIV resistance, and these contradicting reports on its impact on other viruses. It can be stated that CRISPR-Cas 9 mediating editing of the CCR5 gene might have a vital role in curing HIV, but the potential risks should be considered.

6. CRISPR-Cas9 Mediating CXCR4 Gene Editing

The CXCR4 is another chemokine receptor that acts as a co-receptor and is identified next to CCR5, which is involved in CXCR4-tropic HIV strains [34]. The CXCR4 has various biological activities, mainly maintaining the development of hematopoietic stem cells (HSC), and its abnormality correlates with different diseases. Moreover, CXCR4 is utilized by X4 variants of HIV, which mainly target naive and resting CD4 cells, and can be detected in approximately 40–50% of infected individuals over the course of the disease [39]. For example, Hou and Chen [40] have reported that *CXCR4* ablation resulted in resistant of human primary $CD4^+$ T cells against X4 type HIV-1 infection on the cell lines that they have used. The modification occurred when two different sites of the CXCR4 gene were successfully targeted by CRISPR-Cas9 with high specificity and insignificant off-target effects without affecting cell division and propagation [40]. Moreover, in another study, Cas9 from Staphylococcus aureus (SaCas9) and a lentiviral vector have been utilized to disrupt the CXCR4 gene in CD4+ T cell lines, which resulted in expression dysregulation and resistance to HIV-1 infection [41]. It has been reported that a natural CXCR4 mutant (P191A) can abrogate HIV-1 binding to the cell without any negative impact on the differentiation of HSC [42, 43]. A natural mutation in CXCR4, known as CXCR4 P191A mutant, from previous reports has shown inhibition capacity to HIV-1 infection without any negative impact on HSC differentiation. Consequently, Liu and Wang [16] have utilized CRISPR-Cas9 combined with the *piggyBac* transposon technologies to induce the CXCR4 P191A mutant expression in an HIV-1 reporter cell line that resulted in a reduction of HIV-1 replication in biallelic *CXCR4* gene-edited cells, suggesting that individuals who have homologous recombination of the CXCR4 P191A mutant could prevent or reduce HIV-1 infection [16].

The critical function of the CXCR4 protein is that it works as a receptor for CXCL12 (SDF-1), which has a crucial role in T cell functioning and trafficking [44], suggesting that targeting CXCR4 in HIV-1 gene therapy needs to be done more precisely, paying more attention to the safety and side effects as some studies have reported that disruption of CXCR4 resulted in a negative impact on $CD4^+$ T cells viability and functioning [16]. However, studies have shown that *CXCR4* knockout in T lymphocytes

didn't have any abnormal impact in mice, suggesting disruption of *CXCR4* in $CD4^+$ T cells might be tolerable [45, 46]. To sum-up, it can be stated that with precise gene editing, *CXCR4* knockout could be a curative strategy in HIV infection.

Table 1: CRISPR-Cas9-mediated CCR5 and CXCR4 genome editing, creating HIV resistant CD4+ cells.

Targeted Gene	Cell type /Organism	Method	Alteration type and its impact	Contribution	Limitations	Ref
CCR5	TZM-bl cells, CHO-CCR5 cells, and C8166-CCR5 cells	Chimeric Ad5F35 Adenovirus was used as a vector to delivery of CRISPR-Cas9.	The Adenovirus-delivered CRISPR-Cas9 caused CCR5 gene knockout, which resulted in HIV-1 resistance among primary CD4+ T cells.	CRISPR-Cas9 can efficiently edit and knock out CCR5 expression on the cell surface in cell lines, which resulted in primary CD4(+) T resistance to HIV.	-Differences between the efficacy of adenovirus and lentivirus in delivery. - Variability in transduction multiplicity of infection (MOI), integration property, and antibiotic selection.	[37]
CXCR4	HEK293T cells, Ghost-CXCR4 (X4) cells, and Jurkat T cells	T7EN1 assay used to assess mutation	The indel Mutation of the human CXCR4 gene via CRISPR-Cas9, with a few off-targets, resulted in HIV-1 resistance $CD4^+$T cells with no negative impact on cell division and propagation.	The precise genome editing of CXCR4 with an efficient strategy can provide a new avenue in HIV-1 infection therapeutic applications.	- The adenovirus-dependent delivery needs to be optimized to improve specificity and minimize safety concerns.	[40]
CXCR4	HEK293T cell line	A Cas9 from Staphylococcus aureus, along with gRNA, was utilized for editing the genome in mammalian cells.	SaCas9 and selected single-guided RNAs have been transduced using a lentivirus vector to target the CXCR4 gene in human CD4+ T cell lines, causing the cell lines to be resistant to X4-tropic HIV-1 infection.	XCR4 gene modification using SaCas9 improves cell resistance to HIV-1 infection. This provides a possible application of CXCR4-targeted genome editing in	- Low efficacy and toxicity of nucleofection. Moreover, CRISPR-Cas9, with its large size, delays efficient delivery into primary	[41]

		Staphylococcus aureus Cas9 (SaCas9) was used for targeting CXCR4.		HIV-1 gene therapy.	CD4+ T cells.	
CXCR4	TZM-bl cell line	CRISPR-Cas9, along with piggyBac transposon technologies were used to induce the expression of the CXCR4 P191A mutant in an HIV-1 reporter cell line.	A mutation in the CXCR4 gene has resulted in the inhibition of HIV-1 infection. This method didn't leave any genetic footprint; the side effects that might happen due to the destruction of CXCR4 were avoided.	CXCR4 P191A mutation resulted in inhibition of HIV-1 infection with normal HSC differentiation.	NA	[16]
CCR5Δ32/Δ32	human CD4+ Jurkat cell line	Lentiviral packaging system	CRISPR-Cas9 is possible to induce CCR5Δ32/Δ32 homozygotes in CD4+ cells, which can result in CD4+ cells resistant to CCR5-tropic HIV infection.	This approach promises promote HIV/AIDS therapy from the only cured unique Berlin patient to a routine autologous cell-based therapy.	Successful rate of inducing CCR5Δ32/Δ32 is less in primary CD4+ cells	[25]
CCR5	TZM-bl cell line and human T-cell Lymphoma Jurkat (E6-1) cell line	- CCR5 gene targeted by CRISPR-Cas9 mediated homology directed repair (HDR). Double-positive cells were selected by Fluoresce	A biallelic frameshift mutation in the CCR5 gene results in loss of CCR5 expression and inhibition of HIV-1 replication.	The targeted cells, which had a mutation in CCR5, were mediated by CRISPR-Cas9. Showed high resistance to HIV-1 infection.	Week activity shown by gRNA1, no activity at all displayed by gRNA4. Moreover, this strategy introduces lower mutation rates compared to the actual mutation frequency.	[17]

		nce-activated cell sorting (FACS).				
CCR5	TZM-bl cells	Mobilized HSPCs from healthy donors were utilized. Moreover, CD34+CD90+ HSCs were sorted, treated with RUS, and gene-edited.	- CD34+CD90+ HSCs have lower susceptibility to HIV infection. - Gene editing of CD34+CD90+ HSCs provides an ideal strategy for HIV gene therapy.	Editing of the CCR5 gene in CD34+CD90+ HSC cells may offer an ideal gene manipulation strategy for autologous HSCT-based gene therapy for HIV infection.	-The graft quality has limited the clinical application of gene-edited autologous HSPCs. - HIV also infects the HSPCs, limiting their use in therapy.	[47]

7. Simultaneous editing of CCR5 and CXCR4 Mediated by CRISPR-Cas9

As the two strains of HIV-1 (CXCR4- and CCR5-tropic strains) are targeting the various coreceptors, a strategy of dual knockout might have more impact. However, various complications might result from multiplex gene editing; a few studies have shown this method, as summarized in Table 2.

Nonetheless, the possibility of CXCR4 and CCR5 dual knockout in primary human $CD4^+$ T cells has repotted and it was shown that the knockout did not impact cell survival and proliferation [35, 48]. For instance, Liu, Chen [48] has reported efficient simultaneous disruption of both CXCR4 and CCR5 using CRISPR-Cas9 combined with sgRNAs that could target both genes in cell lines and human $CD4^+$ T cells. The $CD4^+$ T cells with disruption showed resistance to X4 and R5 strains of HIV-1 infection. The used sgRNAs were highly specific as they have not resulted in off-targeting. Also, the apoptosis ratio analysis was done to figure out the cytotoxicity impact of the performed disruption, as the result showed no toxicity in primary $CD4^+$ T cells with edited genome suggesting the safety of the performed gene therapy in the future [48].

Another study has also shown the dual ablation of CXCR4 and CCR5 genes in CD4+ cell lines as well as human primary CD4+ T cells using lentivirus delivering system for CRISPR-Cas9 (LV-X4R5) [35]. Consequently, a single LV-X4R5 transduction resulted in ablation of CXCR4 and CCR5, losing a base pair to >10 bps. The nonhomologous end joining (NHEJ), which followed the disruption of targeted genes in modified CD4+ T cells, resulted in resistant for both tropic HIV-1 infections, and in modified GHOST cells, the off-target assay showed the safety of *CXCR4* and *CCR5* ablation for CD4+ T-cell-based HIV-1 therapy [35]. Furthermore, Knipping and Newby [18] have demonstrated a base editing method using CRISPR-Cas9 targeting both CXCR4 and CCR5 without inducing DNA double-strand break resulted in introducing stop codons or eliminating start codons in the targeted genes. Thus, they have shown that utilizing adenine base editors targeting the CCR5 gene resulted in start codon elimination in nearly 95% of primary human $CD4^+$ T cells and 88% of $CD34^+$hematopoietic stem cells, even though a few off-targeting reported in intergenic or intronic regions. This method showed resistance up to 79% and 88% of human $CD4^+$ T cells toward R5 and X4 strains, respectively, Knipping, Newby [18].

In summary, the dual knockout of CXCR4 and CCR5 using CRISPR-Cas9 was found to be feasible in primary human CD4+ T cells and might result in more resistance to HIV-1 as receptors for both X4 and R5 strains are targeted. However, more challenges are expected as technique as well as impacts on cellular activates.

Table 2: Summarizes simultaneous genome editing of both CXCR4 and CCR5 targeting CD4 T cells as a curative strategy for HIV

Targeted Gene	Cell type /Organism	Method	Alteration type and its impact	Contribution	Limitations	Ref
CXCR4 and CCR5	TZM-bl cells, Jurkat T cells, and human $CD4^+$ T cells	CRISPR-Cas9 was used to edit both CXCR4 andCCR5. Moreover, two different gRNAs were designed to target both CXCR4 and CCR5.	Simultaneous genome editing of both CXCR4 and CCR5 would protect CD4+ T cells from HIV infection.	Dual genome modification of CXCR4 and CCR5 using CRISPR-Cas9 might provide a safe and effective strategy to effective HIV-1 cure.	NA	[48]
CXCR4 and CCR5	HEK293T cells, HeLa-CD4-LTR-β-gal cells, and GHOST (3) CXCR4+CCR5+ cells	CRISPR-Cas9 was used to knock out both CXCR4 and CCR5 genes. Lentiviral vector (LV-X4R5) was used to modify the genes in CD4+ cell lines.	CXCR4 and CCR5 are simultaneously knocked out in CD4+ T cells, which results in X4- and R5-tropic resistance to HIV-1 infection.	CRISPR-Cas9 worked as an efficient tool to simultaneous knockout of CXCR4 and CCR5 genes.	NA	[35]
CCR5 and CXCR4	Hematopoietic stem and progenitor cells (HSPCs) and humanized mouse	CCR5 and CXCR4 genes were disturbed using CRISPR-Cas9 in T lymphocyte cells, and CCR5 alleles were disturbed in human CD34+ hematopoietic stem and progenitor cells (HSPCs)	Broad human immunodeficiency virus (HIV) resistance was created by simultaneously disrupting the human CCR5 and CXCR4 genes.	CRISPR-mediated disruption of CCR5 and CXCR4 genes confers broad resistance to HIV infection. - Disruption of CCR5 alleles in CD34+ cells resulted in the differentiation of HIV-resistant macrophages.	- Poor engraftment of CD4+ T cells in bone marrow - Disrupting CXCR4 may abate engraftment of CD4+ T cells	[49]
CCR5 and CXCR4	Human T cells and HSPCs	A base editing strategy, including both cytosine base	The co-receptors (CCR5 and CXCR4) were mutated using a	Along with maintaining functionality, the edited	- Base editing introduced a few off-targeted	[18]

		editors and adenine base editors, was used.	base editing strategy in human T cells and stem cells.	cells showed protection against HIV-1 infection. Moreover, it prevented transduction with CCR5tropic and CXCR4-tropic viral vectors in up to 79% and 88%, respectively.	mutations, as they were located predominantly in intergenic or intronic regions	

8. Targeting of Latent HIV Reservoir

One of the main challenges in curing HIV-1 is that it becomes dormant and forms a latent reservoir, which is replication-competent virus cells. These reservoirs have the ability to cause latent infections that cannot be cleared by antiretroviral drugs or the body's immune responses, as the recently infected cell would not express antigens [50]. Besides, it's been found that latent infected cells are usually located in sites where antiviral drugs might not reach, including the brain, lymphoid tissue, and gastrointestinal tract [19]. The latent viruses mainly get reactivated when the antiviral therapy has been stopped or lapsed; moreover, in case of T cell activation due to stimulus agents, including phorbol 12-myristate 13-acetate, or bryostatin, the latent viruses get reversed as well [51]. The reactivation of latent reservoirs possibly results in presentation of antigens that possibly could be targeted using adoptive therapies [52]. However, the used molecules in adoptive therapy are not suitable to be used in vivo; therefore, genome editing might offer potential to be a curative agent even for the latent reservoir [22].

Targeting, removing, or inactivating the HIV-1 genome inside the infected cells is possible directly by utilizing CRISPR-Cas9. For instance, Zhu, Lei [53] have reported the potency of the CRISPR-Cas9 system in targeting HIV-1 proviral DNA in the latent JLat10.6 cell line. In their study, they have targeted 10 sites and found that one of them (called T10) exhibits the highest level of mutation and inactivation via Cas9. Moreover, another study has reported that CRISPR-Cas9, which has been delivered by Lentivirus targeting the HIV-1 genome, has significantly reduced HIV-1 replication in infected primary CD4+ T-cell cultures, and also resulted in a dramatic reduction of viral load in *ex vivo* culture of CD4+ T-cells obtained from HIV-1-infected patients. Suggesting that this CRISPR-Cas9 dependent therapy eliminated HIV-1 DNA from CD4+ T-cells [54]. Furthermore, Herskovitz, Hasan [55], through in vitro experiments, have demonstrated that targeting the HIV promoter of long terminal repeat in latently infected cells resulted in 100% viral excision.

Targeting the HIV genome has not been applied as a therapeutic strategy yet; the approach is interesting. This approach is different from CCR5/CXCR4 gene editing, as the immune system does not need to be replaced since the viral genome is targeted rather than human genes. Additionally, as the viral genome is targeted, various subtypes of HIV can be targeted with the same method. However, when it comes to in vivo application, various challenges are expected, including cellular heterogenicity, delivering, targeting, and the required dose, as the reservoir size and distribution are variable.

9. Conclusion

Therapeutic approaches for HIV infection have not resulted in curing the disease yet. Thus, CRISPR-Cas9-mediated genome editing as a new and effective avenue might promise a curative strategy for HIV, mainly by targeting coreceptors CCR5 and CXCR4. The precise editing that resulted in CCR5 and CXCR4 knockout in various cell lines, as well as human primary CD4+ T cell resulted in emerging cells with high resistance to HIV-1 Infection. However, to guarantee the in-vivo success, more optimization is required to cause sufficient CRISPR-Cas9 mediated CXCR4 and CCR5 knockout cells. Even though the cases are very few, the naturally occurring CCR5Δ32/32 mutation declares more hope in targeting CCR5 as a promising therapeutic target.

The simultaneous knockout of CXCR4 and CCR5 resulted in resistance to CXCR4- and CCR5-tropic HIV strains. However, the expected challenges in this approach need to be considered, especially as CXCR4 has critical cellular functions. Another method is to target the HIV genome mainly in the long terminal repeat of the latent reservoir, in which this avenue seems to be safer compared to the other approaches, as the human genome does not get targeted; however, this method has more challenges and less success has been reported. On the other hand, the disruption of CCR5/CXCR4 alone or in simultaneous mode would protect patients against reinfection, while in the case of LTR excision, as viral entry would not be blocked, the possibility of reinfection possibility still exists. These disadvantages and benefits need to be carefully studied and considered before implementation in the clinic.

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References

[1] Carbone A, Vaccher E, Gloghini A, Pantanowitz L, Abayomi A, De Paoli P, et al. Diagnosis and management of lymphomas and other cancers in HIV-infected patients. Nature reviews Clinical oncology. 2014;11(4):223-38. doi:https://doi.org/10.1038/nrclinonc.2014.31

[2] Chen LF, Hoy J, Lewin SR. Ten years of highly active antiretroviral therapy for HIV infection. Medical Journal of Australia. 2007;186(3):146-51.

[3] Andrade HB, Shinotsuka CR, da Silva IRF, Donini CS, Yeh Li H, de Carvalho FB, et al. Highly active antiretroviral therapy for critically ill HIV patients: a systematic review and meta-analysis. PLoS One. 2017;12(10):e0186968. doi:https://doi.org/10.1371/journal.pone.0186968

[4] Costiniuk CT, Jenabian M-A. HIV reservoir dynamics in the face of highly active antiretroviral therapy. AIDS patient care and STDs. 2015;29(2):55-68. doi:https://doi.org/10.1089/apc.2014.0173

[5] Vidya Vijayan K, Karthigeyan KP, Tripathi SP, Hanna LE. Pathophysiology of CD4+ T-cell depletion in HIV-1 and HIV-2 infections. Frontiers in immunology. 2017;8:580. doi:https://doi.org/10.3389/fimmu.2017.00580

[6] Chen B. Molecular mechanism of HIV-1 entry. Trends in microbiology. 2019;27(10):878-91. doi:https://doi.org/10.1016/j.tim.2019.06.002

[7] Connor RI, Sheridan KE, Ceradini D, Choe S, Landau NR. Change in coreceptor use correlates with disease progression in HIV-1–infected individuals. The Journal of Experimental Medicine. 1997;185(4):621-8. doi:https://doi.org/10.1084%2Fjem.185.4.621

[8] Lim JK, McDermott DH, Lisco A, Foster GA, Krysztof D, Follmann D, et al. CCR5 deficiency is a risk factor for early clinical manifestations of West Nile virus infection but not for viral transmission. The Journal of Infectious Diseases. 2010;201(2):178-85. doi:https://doi.org/10.1086/649426

[9] Didigu CA, Wilen CB, Wang J, Duong J, Secreto AJ, Danet-Desnoyers GA, et al. Simultaneous zinc-finger nuclease editing of the HIV coreceptors ccr5 and cxcr4 protects CD4+ T cells from HIV-1 infection. Blood, The Journal of the American Society of Hematology. 2014;123(1):61-9. doi:https://doi.org/10.1182/blood-2013-08-521229

[10] Jiang F, Doudna JA. CRISPR–Cas9 structures and mechanisms. Annual review of biophysics. 2017;46:505-29. doi:https://doi.org/10.1146/annurev-biophys-062215-010822

[11] Niu Y, Shen B, Cui Y, Chen Y, Wang J, Wang L, et al. Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one-cell embryos. Cell. 2014;156(4):836-43. doi:https://doi.org/10.1016/j.cell.2014.01.027

[12] Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, Mikkelsen TS, et al. Genome-scale CRISPR-Cas9 knockout screening in human cells. Science. 2014;343(6166):84-7. doi:https://doi.org/10.1126/science.1247005

[13] Martinez-Lage M, Puig-Serra P, Menendez P, Torres-Ruiz R, Rodriguez-Perales S. CRISPR/Cas9 for cancer therapy: hopes and challenges. Biomedicines. 2018;6(4):105. doi:https://doi.org/10.3390/biomedicines6040105

[14] Mohammadzadeh I, Qujeq D, Yousefi T, Ferns GA, Maniati M, Vaghari-Tabari M. CRISPR/Cas9 gene editing: A new therapeutic approach in the treatment of infection and autoimmunity. IUBMB life. 2020;72(8):1603-21. doi:https://doi.org/10.1002/iub.2296

[15] Dhanda S. Applications of CRISPR/Cas9 as a Tool to study Human Diseases caused by Viruses: A review. 2023.

[16] Liu S, Wang Q, Yu X, Li Y, Guo Y, Liu Z, et al. HIV-1 inhibition in cells with a CXCR4 mutant genome created by CRISPR-Cas9 and piggyBac recombinant technologies. Scientific Reports. 2018;8(1):1-11. doi:https://doi.org/10.1038/s41598-018-26894-4

[17] Scheller SH, Rashad Y, Saleh FM, Willingham KA, Reilich A, Lin D, et al. Biallelic, selectable, knock-in targeting of CCR5 via CRISPR-Cas9 mediated homology directed repair inhibits HIV-1 replication. Frontiers in immunology. 2022;13:821190. doi:https://doi.org/10.3389/fimmu.2022.821190

[18] Knipping F, Newby GA, Eide CR, McElroy AN, Nielsen SC, Smith K, et al. Disruption of HIV-1 co-receptors CCR5 and CXCR4 in primary human T cells and hematopoietic stem and progenitor cells using base editing. Molecular Therapy. 2022;30(1):130-44. doi:https://doi.org/10.1016/j.ymthe.2021.10.026

[19] Huang Z, Tomitaka A, Raymond A, Nair M. Current application of CRISPR/Cas9 gene-editing technique to eradication of HIV/AIDS. Gene therapy. 2017;24(7):377-84. doi:https://doi.org/10.1038/gt.2017.35

[20] Boasso A, Shearer G, Chougnet C. Immune dysregulation in human immunodeficiency virus infection: know it, fix it, prevent it? Journal of Internal Medicine. 2009;265(1):78-96. doi:https://doi.org/10.1111/j.1365-2796.2008.02043.x

[21] Aiken C, Rousso I. The HIV-1 capsid and reverse transcription. Retrovirology. 2021;18(1):1-9. doi:https://doi.org/10.1186/s12977-021-00566-0

[22] Freen-van Heeren JJ. Closing the Door with CRISPR: Genome Editing of CCR5 and CXCR4 as a Potential Curative Solution for HIV. BioTech. 2022;11(3):25. doi:https://doi.org/10.3390/biotech11030025
[23] Himmel DM, Arnold E. Non-nucleoside reverse transcriptase inhibitors join forces with integrase inhibitors to combat HIV. Pharmaceuticals. 2020;13(6):122. doi:https://doi.org/10.3390/ph13060122
[24] Voshavar C. Protease inhibitors for the treatment of HIV/AIDS: recent advances and future challenges. Current Topics in Medicinal Chemistry. 2019;19(18):1571-98. doi:https://doi.org/10.2174/1568026619666190619115243
[25] Qi C, Li D, Jiang X, Jia X, Lu L, Wang Y, et al. Inducing CCR5Δ32/Δ32 homozygotes in the human Jurkat CD4+ cell line and primary CD4+ cells by CRISPR-Cas9 genome-editing technology. Molecular Therapy-Nucleic Acids. 2018;12:267-74. doi:https://doi.org/10.1016/j.omtn.2018.05.012
[26] Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014;346(6213):1258096. doi:https://doi.org/10.1126/science.1258096
[27] Gasiunas G, Barrangou R, Horvath P, Siksnys V. Cas9–crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proceedings of the National Academy of Sciences. 2012;109(39):E2579-E86. doi:https://doi.org/10.1073/pnas.1208507109
[28] Jinek M, East A, Cheng A, Lin S, Ma E, Doudna J. RNA-programmed genome editing in human cells. elife. 2013;2:e00471. doi:https://doi.org/10.7554/eLife.00471
[29] Redman M, King A, Watson C, King D. What is CRISPR/Cas9? Archives of Disease in Childhood-Education and Practice. 2016;101(4):213-5. doi:https://doi.org/10.1136/archdischild-2016-310459
[30] Liu Z, Dong H, Cui Y, Cong L, Zhang D. Application of different types of CRISPR/Cas-based systems in bacteria. Microbial cell factories. 2020;19(1):1-14. doi:https://doi.org/10.1186/s12934-020-01431-z
[31] Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, et al. RNA-guided human genome engineering via Cas9. Science. 2013;339(6121):823-6. doi:https://doi.org/10.1126/science.1232033
[32] Guilinger JP, Thompson DB, Liu DR. Fusion of catalytically inactive Cas9 to FokI nuclease improves the specificity of genome modification. Nature Biotechnology. 2014;32(6):577-82. doi:https://doi.org/10.1038/nbt.2909
[33] Gupta RM, Musunuru K. Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9. The Journal of Clinical Investigation. 2014;124(10):4154-61. doi:https://doi.org/10.1172/JCI72992
[34] Moore JP, Kitchen SG, Pugach P, Zack JA. The CCR5 and CXCR4 coreceptors—central to understanding the transmission and pathogenesis of human immunodeficiency virus type 1 infection. AIDS research and human retroviruses. 2004;20(1):111-26. doi:https://doi.org/10.1089/088922204322749567
[35] Yu S, Yao Y, Xiao H, Li J, Liu Q, Yang Y, et al. Simultaneous knockout of CXCR4 and CCR5 genes in CD4+ T cells via CRISPR/Cas9 confers resistance to both X4-and R5-tropic human immunodeficiency virus type 1 infection. Human gene therapy. 2018;29(1):51-67. doi:https://doi.org/10.1089/hum.2017.032
[36] Hütter G, Nowak D, Mossner M, Ganepola S, Müßig A, Allers K, et al. Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. New England Journal of Medicine. 2009;360(7):692-8. doi:https://doi.org/10.1056/NEJMoa0802905

[37] Li C, Guan X, Du T, Jin W, Wu B, Liu Y, et al. Inhibition of HIV-1 infection of primary CD4+ T-cells by gene editing of CCR5 using adenovirus-delivered CRISPR/Cas9. Journal of General Virology. 2015;96(8):2381-93. doi:https://doi.org/10.1099/vir.0.000139

[38] Ellwanger JH, Kulmann-Leal B, de Lima Kaminski V, Rodrigues AG, de Souza Bragatte MA, Chies JAB. Beyond HIV infection: neglected and varied impacts of CCR5 and CCR5Δ32 on viral diseases. Virus research. 2020;286:198040.

[39] Brumme ZL, Goodrich J, Mayer HB, Brumme CJ, Henrick BM, Wynhoven B, et al. Molecular and clinical epidemiology of CXCR4-using HIV-1 in a large population of antiretroviral-naive individuals. The Journal of Infectious Diseases. 2005;192(3):466-74. doi:https://doi.org/10.1086/431519

[40] Hou P, Chen S, Wang S, Yu X, Chen Y, Jiang M, et al. Genome editing of CXCR4 by CRISPR/Cas9 confers cells resistant to HIV-1 infection. Scientific reports. 2015;5(1):15577. doi:https://doi.org/10.1038/srep15577

[41] Wang Q, Chen S, Xiao Q, Liu Z, Liu S, Hou P, et al. Genome modification of CXCR4 by Staphylococcus aureus Cas9 renders cells resistance to HIV-1 infection. Retrovirology. 2017;14:1-12.

[42] Tian S, Choi W-T, Liu D, Pesavento J, Wang Y, An J, et al. Distinct functional sites for human immunodeficiency virus type 1 and stromal cell-derived factor 1α on CXCR4 transmembrane helical domains. Journal of Virology. 2005;79(20):12667-73. doi:https://doi.org/10.1128/jvi.79.20.12667-12673.2005

[43] Liu Y, Zhou J, Pan J-A, Mabiala P, Guo D. A novel approach to block HIV-1 coreceptor CXCR4 in a non-toxic manner. Molecular biotechnology. 2014;56:890-902. doi:https://doi.org/10.1007/s12033-014-9768-7

[44] Schumann K, Lin S, Boyer E, Simeonov DR, Subramaniam M, Gate RE, et al. Generation of knock-in primary human T cells using Cas9 ribonucleoproteins. Proceedings of the National Academy of Sciences. 2015;112(33):10437-42. doi:https://doi.org/10.1073/pnas.1512503112

[45] Xie F, Ye L, Chang JC, Beyer AI, Wang J, Muench MO, et al. Seamless gene correction of β-thalassemia mutations in patient-specific iPSCs using CRISPR/Cas9 and piggyBac. Genome research. 2014;24(9):1526-33. doi:http://www.genome.org/cgi/doi/10.1101/gr.173427.114

[46] Zhu S, Rong Z, Lu X, Xu Y, Fu X. Gene targeting through homologous recombination in monkey embryonic stem cells using CRISPR/Cas9 system. Stem cells and development. 2015;24(10):1147-9. doi:https://doi.org/10.1089/scd.2014.0507

[47] Karuppusamy KV, Demosthenes JP, Venkatesan V, Christopher AC, Babu P, Azhagiri MK, et al. The CCR5 gene-edited CD34+ CD90+ hematopoietic stem cell population serves as an optimal graft source for HIV gene therapy. Frontiers in Immunology. 2022;13:792684. doi:https://doi.org/10.3389/fimmu.2022.792684

[48] Liu Z, Chen S, Jin X, Wang Q, Yang K, Li C, et al. Genome editing of the HIV co-receptors CCR5 and CXCR4 by CRISPR-Cas9 protects CD4+ T cells from HIV-1 infection. Cell & bioscience. 2017;7:1-15. doi:https://doi.org/10.1186/s13578-017-0174-2

[49] Li S, Holguin L, Burnett JC. CRISPR-Cas9-mediated gene disruption of HIV-1 co-receptors confers broad resistance to infection in human T cells and humanized mice. Molecular Therapy-Methods & Clinical Development. 2022;24:321-31. doi:https://doi.org/10.1016/j.omtm.2022.01.012

[50] Llewellyn GN, Seclén E, Wietgrefe S, Liu S, Château M, Pei H, et al. Humanized mouse model of HIV-1 latency with enrichment of latent virus in PD-1+ and TIGIT+ CD4 T cells. Journal of Virology. 2019;93(10):10.1128/jvi. 02086-18. doi:https://doi.org/10.1128/jvi.02086-18

[51] Barton K, Winckelmann A, Palmer S. HIV-1 reservoirs during suppressive therapy. Trends in microbiology. 2016;24(5):345-55. doi:https://doi.org/10.1016/j.tim.2016.01.006
[52] Freen-van Heeren JJ. Addressing HIV-1 latency with flow-FISH: finding, characterizing, and targeting HIV-1 infected cells. Wiley Online Library; 2021. p. 861-5.
[53] Zhu W, Lei R, Le Duff Y, Li J, Guo F, Wainberg MA, et al. The CRISPR/Cas9 system inactivates latent HIV-1 proviral DNA. Retrovirology. 2015;12:1-7. doi:https://doi.org/10.1186/s12977-015-0150-z
[54] Kaminski R, Chen Y, Fischer T, Tedaldi E, Napoli A, Zhang Y, et al. Elimination of HIV-1 genomes from human T-lymphoid cells by CRISPR/Cas9 gene editing. Scientific reports. 2016;6(1):22555. doi:https://doi.org/10.1038/srep22555
[55] Herskovitz J, Hasan M, Patel M, Blomberg WR, Cohen JD, Machhi J, et al. CRISPR-Cas9-mediated exonic disruption for HIV-1 elimination. EBioMedicine. 2021;73. doi:https://doi.org/10.1016/j.ebiom.2021.103678